Global Change Biology

Increased soil carbon storage through plant diversity strengthens with time and extends into the subsoil

Abstract:	Soils are important for ecosystem functioning and service provisioning. Soil communities and their functions, in turn, are strongly promoted by plant diversity, and such positive effects strengthen with time. However, plant diversity effects on soil organic matter have mostly been investigated in the topsoil, and there are only very few long-term studies. Thus, it remains unclear if plant diversity effects strengthen with time and to which depth these effects extend. Here, we repeatedly sampled soil to one-meter depth in a long-term grassland biodiversity experiment. We investigated how plant diversity impacted soil organic carbon and nitrogen concentrations and stocks and their stable isotopes 13C and 15N, as well as how these effects changed after five, ten, and 14 years. We found that higher plant diversity increased carbon and nitrogen storage in the topsoil since the establishment of the experiment. Stable isotopes revealed that this increase was associated with new plant-derived inputs, resulting in less processed and less decomposed soil organic matter. In subsoils, mainly the presence of specific plant functional groups drove organic matter dynamics. For example, the presence of deep-rooting tall herbs decreased carbon concentrations, most probably through stimulating soil organic matter decomposition. Moreover, plant diversity effects on soil organic matter became stronger in topsoil over time and reached subsoil layers, while the effects of specific plant functional groups in subsoil progressively diminished over time. Our results indicate that after changing the soil system the pathways of organic matter transfer to the subsoil need time to establish. In our grassland system, organic matter storage in subsoils was driven by the redistribution of already stored soil organic matter from the topsoil to deeper soil layers, e.g., via bioturbation or dissolved organic matter. Therefore, managing plant diversity may thus have significant implications for subsoil carbon storage and other critical

	ecosystem services.

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Running title: Diversity effects on subsoil OM storage

1 **Increased soil carbon storage through plant diversity strengthens with time**

2 **and extends into the subsoil**

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Abstract

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15   their functions, in turn, are strongly promoted by plant diversity, and such positive effects strengthen

16   with time. However, plant diversity effects on soil organic matter have mostly been investigated in the

17   topsoil, and there are only very few long-term studies. Thus, it remains unclear if plant diversity effects

18   strengthen with time and to which depth these effects extend. Here, we repeatedly sampled soil to

19   one-meter depth in a long-term grassland biodiversity experiment. We investigated how plant

20   diversity impacted soil organic carbon and nitrogen concentrations and stocks and their stable isotopes

21   ^{13}C and ^{15}N, as well as how these effects changed after five, ten, and 14 years. We found that higher

22   plant diversity increased carbon and nitrogen storage in the topsoil since the establishment of the

23   experiment. Stable isotopes revealed that this increase was associated with new plant-derived inputs,

24   resulting in less processed and less decomposed soil organic matter. In subsoils, mainly the presence

25   of specific plant functional groups drove organic matter dynamics. For example, the presence of deep-

26   rooting tall herbs decreased carbon concentrations, most probably through stimulating soil organic

27   matter decomposition. Moreover, plant diversity effects on soil organic matter became stronger in

28   topsoil over time and reached subsoil layers, while the effects of specific plant functional groups in

29   subsoil progressively diminished over time. Our results indicate that after changing the soil system the

30   pathways of organic matter transfer to the subsoil need time to establish. In our grassland system,

31   organic matter storage in subsoils was driven by the redistribution of already stored soil organic matter

32   from the topsoil to deeper soil layers, e.g., via bioturbation or dissolved organic matter. Therefore,

33   managing plant diversity may thus have significant implications for subsoil carbon storage and other

34   critical ecosystem services.

Keywords

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Introduction

38   Plant diversity increases ecosystem functions and services (Cardinale et al., 2012; Isbell et al.,

39   2011). This positive biodiversity–ecosystem functioning (BEF) relationship is particularly important for

40   belowground processes, such as soil organic carbon (C) and nutrient cycles and storage, as well as soil

41   fertility (Hooper et al., 2012), which then feedback on ecosystem productivity (Wardle et al., 2004).

42   This positive BEF relationship even strengthens over time in experimental settings (Cardinale et al.,

43   2007; Eisenhauer et al., 2012; Guerrero-Ramírez et al., 2017; Reich et al., 2012). However, most studies

44   investigating C storage and nutrient cycling in the BEF context focus on topsoil (Cong et al., 2014; Lange

45   et al., 2015), cover a relatively short period, often less than five years (e.g., Steinbeiss et al., 2008; Xu

46   et al., 2020), or consider C storage and nutrient cycling over longer periods, as processes in are steady

47   state (but see Yang et al., 2019). There are only a few long-term studies examining the BEF relationships

48   in the topsoil (Furey & Tilman, 2021; Lange et al., 2019). Studies on the long-term plant diversity effects

49   on C and nitrogen (N) dynamics in the subsoil are even more rare. Thus, the temporal dynamics and

50   the depth extent of the effects of plant diversity and community composition on the soil organic matter

51   and its key components (C and N) have been rarely, or not at all, considered so far.

52   The main sources of soil organic matter in natural terrestrial systems are leaf litter inputs to

53   the soil surface and root-derived inputs (Amundson, 2001; Jobbagy & Jackson, 2000). The distributions

54   of root and soil organic matter are highly correlated and decline exponentially with soil depth (Jobbagy

55   & Jackson, 2000). Diversity and composition of plant communities strongly impact the production of

56   shoots and roots (Marquard et al., 2009; Mueller et al., 2013; Ravenek et al., 2014; Tilman et al., 2001)

57   and thus, the fresh plant-derived C inputs into the soil (Eisenhauer et al., 2017; Mellado-Vazquez et al.,

58   2016). It is assumed that in more diverse plant mixtures roots grow denser and to deeper soil layers

59   (Fargione & Tilman, 2005; Mommer et al., 2010; Mueller et al., 2013), potentially impacting the cycling

60   of organic C and N in the subsoil. However, whether diversity and rooting depth of the plant

61   community are related to each other likely depend on environmental factors, such as the presence of

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62   legumes or the fertility of soils. Specifically, in the N-limited, sandy soil of the Cedar Creek biodiversity

63   experiment high plant diversity led to increased root biomass deeper in the soil profile (Mueller et al.,

64   2013), while no significant plant diversity effects were found on the root distribution in the soil profile

65   of the fertile soil of the Jena Experiment (Ravenek et al., 2014).

66   Plant-derived C inputs fuel the soil microbial community, with higher plant diversity leading to

67   a more abundant, more diverse, and more active microbial community (Eisenhauer et al., 2010; Lange

68   et al., 2015; Lange et al., 2014; Zak et al., 2003). The microbial community, in turn, respires major

69   amounts of the plant-derived C but also converts the easily decomposable plant C into forms that are

70   better retained in soil, such as metabolic end products and microbial necromass (Bradford et al., 2013;

71   Gleixner, 2013; Lange et al., 2015; Liang et al., 2019). While consistent positive effects of plant diversity

72   on soil C and N storage were reported for upper soil layers (e.g. De Deyn et al., 2011; Fornara & Tilman,

73   2008; Steinbeiss et al., 2008), subsoil C and N stocks are suggested to be stable with slow element

74   turnover. This is in line with the depletion of modern C in soil organic matter of deeper soil layers

75   (Trumbore, 2009). However, soil organic matter in deeper soil layers is very heterogeneous with

76   patches having accelerated elemental cycling, which are related to rhizodeposition and the activity of

77   the soil fauna (Kuzyakov & Blagodatskaya, 2015).

78   It has been proposed that roots contribute to soil organic matter sequestration in subsoils

79   (Balesdent & Balabane, 1996; Rasse et al., 2005) and that plant diversity and increased root production

80   lead to more storage of C and N down to 60 cm soil depth (Fornara & Tilman, 2008). On the other

81   hand, fresh and labile compounds exuded by roots may stimulate the decomposition of existing soil

82   organic matter in subsoils, causing the so-called 'priming effect' (Fontaine et al., 2007; Kuzyakov,

83   2010). In addition to plant species richness, particular plant functional groups and their composition

84   within the plant community are assumed to impact soil element cycling in different layers of the soil

85   profile (Fornara & Tilman, 2008) as for instance the roots of tall herbs reach much deeper soil layers

86   than those of grasses (Ebeling et al., 2014).

87   Additionally, the quality of root inputs (e.g. C/N ratios) is affected by plant diversity and plant

88   functional group composition, in particular by the presence of legumes (Bessler et al., 2012). In turn,

89   the quality of plant-derived organic matter influences its decomposition (Chen et al., 2017). For

90   example, root turnover is higher in herbs than in grasses, which putatively increases the C and N inputs

91   to the soil (Fornara & Tilman, 2009). This in turn results in increased N availability in soils (Oelmann et

92   al., 2007; Spehn et al., 2005) with potential effects on the microbial transformation of soil organic

93   matter (Lange et al., 2019; Leimer et al., 2016). However, so far it is not clear how plant diversity

94   impacts subsoil dynamics of C and N. For instance, do the enhanced plant C inputs to subsoil with

95   higher diversity lead to more C sequestration or to more C losses due to the priming effect?

96   Furthermore, it is unclear if higher plant diversity leads to more N exploitation of the deeper soil layers

97   due to higher nutrient demands of more diverse and more productive plant communities, or if plant

98   diversity influences the soil nutrient retention via other mechanisms, e.g. related to soil microbial

99   communities and activity (Leimer et al., 2016).

100   For investigating processes and dynamics of soil organic matter, it is insightful to assess C and

101   N changes together with their stable isotopes ^{13}C and ^{15}N, respectively (Balesdent & Balabane, 1996;

102   Ehleringer et al., 2000). Generally, soil organic matter is enriched in the ^{13}C and ^{15}N signatures (increase

103   in $\delta^{13}C$ and $\delta^{15}N$) compared to plant material due to fractionation during microbial mineralisation and

104   processing of organic C and N (Hobley et al., 2017; Högberg, 1997; Kramer et al., 2003; Nadelhoffer &

105   Fry, 1988). This causes an increased enrichment of ^{13}C and ^{15}N with soil depth, where a higher

106   mineralization rate and more processed soil organic matter can be found (Acton et al., 2013; Balesdent

107   et al., 1993; Garten Jr., 2006). However, it is uncertain how plant diversity and plant community

108   composition affect the isotopic composition of soil organic matter below the topsoil. Increased plant

109   diversity might affect the dynamics of stable isotopes in both directions: enrichment due to a more

110   active microbial community or depletion due to more fresh plant-derived inputs. The combined

111   assessment of the concentration of C or N together with their respective stable isotopes enables to

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112   draw conclusions on processes that take place and impact changes in the soil organic matter (Nel et

113   al., 2018). For instance, an increase of the C concentrations accompanied with a depletion of ^{13}C

114   (decrease in δ^{13}C values) indicates that input of fresh plant material mainly contributes to soil organic

115   matter formation and sequestration. In contrast, the decrease of C concentrations and the enrichment

116   of ^{13}C indicates enhanced decomposition and loss of soil organic matter, while the decrease of C

117   concentrations and the depletion of ^{13}C indicates priming of stored soil organic C and its replacement

118   with fresh plant-derived C.

119   This study investigated the effects of plant diversity and plant functional group composition

120   on C and N storage and cycling within the soil profile down to one meter. Taking advantage of a long-

121   term grassland biodiversity experiment, the Jena Experiment, we were able to assess the effects of the

122   plant diversity and functional group composition on the changes of soil organic matter in regular

123   intervals over 14 years. Specifically we asked i) whether the stocks, concentrations and stable isotope

124   ratios of organic C and N changed similarly in depth segments of the soil profile after the conversion

125   from an arable land to a grassland; ii) whether the effects of plant diversity and/or functional group

126   composition on the stocks, concentrations and stable isotope ratios of organic C and N were similar in

127   the subsoil and the topsoil; iii) whether plant community effects strengthened over time; and iv) what

128   processes determined the changes of soil organic C and N stocks, concentrations and stable isotope

129   ratios?

Materials and Methods

Study site of The Jena Experiment

132   This study was carried out as a part of The Jena Experiment, a large-scale grassland diversity

133   experiment (Roscher et al., 2004; Weisser et al., 2017). The Jena Experiment is located on the

134   floodplain of the Saale River near the city of Jena (Thuringia, Germany; 50°57´N, 11°35´E). The soil of

135   the field site is classified as Eutric Fluvisol (FAO-UNESCO, 1997), developed from loamy fluvial

136   sediments. The texture ranged from sandy loam to silty clay with increasing distance to the river Saale

137   reflecting the sedimentation process. For 40 years prior to the establishment of the experiment, the

138   field site was a cropland with inputs of mineral fertilizer. In spring 2002, 82 experimental grassland

139   plots with a size of 20 × 20 m were established. Plots are arranged in four blocks to account for changes

140   in soil texture. In this study, we focused on the most intensively investigated block 2. While initial soil

141   pH was similar among plots (7.2 - 7.4), soil texture in the top 20 cm varied within block 2. Specifically,

142   sand portion increased in the upper 20 cm of the soil profile from 10% in the north to 38% in the south

143   of the block, while silt decreased from 70 to 44%. Clay showed almost no spatial trend and was in the

144   range of 17 to 27%. In contrast, in 20 - 100 cm depth, soil texture was homogenous containing 16%

145   sand, 59% silt, and 25% clay.

146   The experimental plots were assembled by randomly selecting plant species from the 60

147   species pool with consideration of species richness and functional group richness and composition.

148   The experimental plots spread gradients of plant species richness from 1 to 60 (i.e., 1, 2, 4, 8, 16, and

149   60) and the plant functional group richness from 1 to 4, including grasses, legumes, small herbs, and

150   tall herbs based on morphological, phenological, and physiological traits; for details see (Roscher et

151   al., 2004). The block 2 contained four replicates of monocultures, 2-, 4-, and 8-species mixtures as well

152   as three replicates of 16-species mixtures (n=19). Experimental communities are weeded manually

153   twice to three times per year to maintain the plant diversity levels and community composition, and

154   mown twice per year in June and September, as is typical for hay meadows in Central Europe.

155   **Soil sampling and laboratory analysis**

156   Soil samples were taken in April 2002 before sowing and five, ten and 14 years after in April of

157   each sampling year. Three independent 1-m soil cores per plot were collected using a machine-driven

158   soil corer (Cobra, Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) with an inner diameter

159   of 8.7 cm. Soil cores were segmented into 5 cm sections, resulting in 20 segments per soil core. Soil

160 samples were air-dried sieved to 2 mm and milled. After each sampling subsamples were analysed for

161 organic C and total N concentrations with a Vario Max and a Vario EL (Elementar Analysensysteme

162 GmbH, Hanau, Germany), respectively. Organic C was determined as the difference between the total

163 C concentration and the inorganic C concentration; the latter was measured after removing the organic

164 C by heating the sample to 450°C for 16 h in a muffle furnace. This method is highly reliable (Steinbeiss

165 et al., 2008) and even outperformed methods using acids to remove the inorganic C in terms of

166 accuracy in our laboratory (Bisutti et al., 2004; Schreider-Goidenko, 2015). In 2002, soil bulk density

167 was determined at 6 plots on block 2. The depth segments for density measurements ranged from 0 -

168 10 cm, 10 - 20 cm, 20 - 30 cm, 30 - 40 cm, 40 - 60 cm, 60 - 80 cm, to 80 - 100 cm. Samples were taken

169 with a metal bulk density ring of 10 cm height, sieved to 2 mm, and dried at 105 °C. Soil density was

170 calculated by weight (Hartge & Horn, 1992). The chosen plots represented a spatial gradient across the

171 block and resulted in an average soil bulk density value at the beginning of the experiment. In the later

172 soil sampling campaigns in 2007, 2012 and 2016, changes in bulk density were measured for every plot

173 in block 2 with 5 cm depth resolution using the inner diameter of the soil corer for volume calculation.

174 To adapt the depth resolution of 2002 to the later sampling campaigns in 0 - 30 cm depth a logarithmic

175 and in 30 - 100 cm depth a linear regression was applied ($0.8 \leq R \leq 1$ and see Table S1).

176 Further $\delta^{13}C$ values of organic C and $\delta^{15}N$ values of total N were measured with a DeltaPlus

177 isotope ratio mass spectrometer (Thermo Fisher, Bremen, Germany), coupled via a ConFlowIII open-

178 split to an elemental analyser (Carlo Erba 1100 CE analyser; Thermo Fisher Scientific, Rodano, Italy)

179 after carbonates in soil being removed with 120 ml of 5 - 6% H_2SO_3 (Steinbeiss et al., 2008).

180 To calculate the changes in the concentrations and stocks of organic C and total N as well as

181 changes in the $\delta^{13}C$ values (= $\Delta\delta^{13}C$) and $\delta^{15}N$ values (= $\Delta\delta^{15}N$) over time, values measured in 2002 were

182 averaged first for each depth session of each plot and subtracted from the values measured per core

183 and layer in the later years (2007, 2012, and 2016), resulting in periods of 5, 10 and 14 years. Similarly,

184   we calculated the mean annual changes in organic C and total N stocks and concentrations as well as

185    $\delta^{13}C$ values, $\delta^{15}N$ values, and C:N ratios for each sampling period.

Statistical analyses

187   All statistical analyses were conducted with the statistical software R (2018). Linear mixed-

188   effects models (LMM) applying the 'lme'-function in the R library 'nlme' (Pinheiro et al., 2016) were

189   applied to test for plant diversity effects and their changes over time on the concentrations and stocks

190   of C, N, their isotopic values ($\delta^{13}C$, $\delta^{15}N$) and the C/N ratios. Starting from a constant null model, with

191   plot identity as random effect, sown plant species richness (log-linear term) was fitted first, followed

192   by plant functional groups richness (linear term); and in alternative models the presence of all

193   individual plant functional groups was fitted, as they are not independent of each other (Roscher et

194   al., 2004). The maximum likelihood method and likelihood ratio tests were applied to assess the

195   statistical significance of stepwise model improvement. The effect size of every tested variable was

196   determined as marginal R-squared (i.e. the effect size of the random effect plot identity was not

197   considered) using the 'r.squaredGLMM'-function in the R library 'MuMIn' (Bartoń, 2013). Plant species

198   richness, functional groups richness, and the presence of the functional groups were varied as

199   orthogonally as possible in the experimental design (Roscher et al., 2004). To account for the possible

200   correlations between plant species richness and functional group richness, and to test which of the

201   both factors was more important, all models were run twice with a changed sequence of fitting, i.e.,

202   plant species richness vs. functional group richness (Eisenhauer et al., 2010). Here we reported the

203   results of the model that explained best the variance of the response variables. In most models, plant

204   species richness was a better predictor in comparison to functional group richness when fitted first.

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Results

**Soil organic matter changes within the soil profile after land use change**

In 2002, before the grassland was established, soil organic matter was homogeneously distributed within the ploughing horizon: concentrations of C and N, the C/N ratio, as well as the isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ were at similar levels among soil sections and plots in the upper 30 cm (Fig. 1; average values of C: 20.9±2.6 g kg^{-1} (mean±SD), N: 2.2±0.2 g kg^{-1}, C/N ratio: 9.4±0.4, $\delta^{13}C$: -26.8±0.3‰, $\delta^{15}N$: 6.3±0.4‰). Accordingly, the decrease of the C and N stocks in the first 20 cm of the soil profile was driven by increased bulk density with increasing soil depth. Below the plough horizon, concentrations and stocks of C and N strongly decreased between 30 and 45 cm, followed by a moderate decrease, resulting in very low concentrations of C and N at one-meter soil depth (C: 6.7±1.4 g kg^{-1}, N: 0.8±0.2 g kg^{-1}). Below the plough horizon, ^{13}C and ^{15}N became slightly enriched with increasing soil depth, up to values of $\delta^{13}C$: -25.6±0.3‰ and $\delta^{15}N$: 6.8±0.3‰ at one-meter depth (Fig. 1).

After 14 years since the establishment of the Jena Experiment, soil organic matter has developed towards a typical grassland soil profile; i.e. in particular in the top 5 cm, C and N concentrations strongly increased (C: +6.4±4.2 g kg^{-1}, N: +0.6±0.4 g kg^{-1}, Fig 1), whereas the concentrations largely decreased in soil sections between 10 and 40 cm (C: -1.9±2.7 g kg^{-1}, N: -0.1±0.3g kg^{-1}, Fig. 1). The decrease of the C and N stocks was less pronounced, but significant between 10 and 40 cm soil depth (C: -1.8±52.0 g m^{-2}, N: -0.8±5.0 g m^{-2}). Thus, the C and N stock gains in the top 10 cm of the soil (C: 9.6±25.3 g m^{-2}, N: 0.9±2.8 g m^{-2}) exceed the losses below that soil depth. Below 40 cm, the changes in C and N stocks and concentrations were marginal (Fig. 1) but still significant for C concentrations in most subsoil depth layers and for N stocks down to a depth of 80 cm.

After 14 years, the ^{13}C values and ^{15}N values were strongly depleted in the uppermost 5 cm ($\Delta\delta^{13}C$: -0.9±0.5‰, $\Delta\delta^{15}N$: -1.2±0.6‰). Below a soil depth of 10 cm, $\delta^{13}C$ values did not change

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228   considerably, and ^{15}N got slightly enriched till a depth of 80 cm. Below 80 cm, the enrichment in [15]N

229   increased, being highest at one-meter soil depth ($\Delta\delta^{15}$N: 0.3±0.5‰, Fig. 1).

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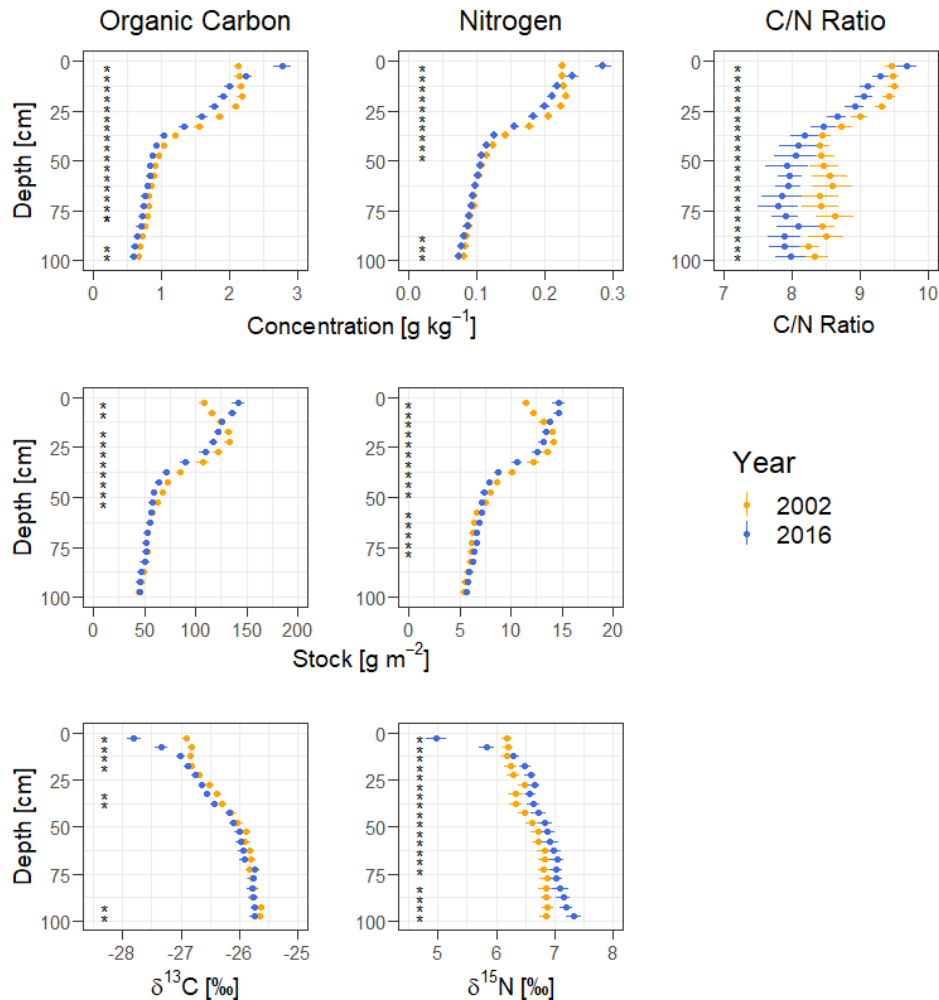
Figure 1: Depth profiles of organic carbon and nitrogen concentrations and stocks, their stable isotope ratios δ^{13}C and δ^{15}N
values, as well as the carbon-to-nitrogen ratio (C/N ratio) before the experimental field site was established in 2002 (yellow)
and 14 years later in 2016 (blue). Circles through the depth profile represent the means, and the error bars show 2x the
standard error of the mean. Asterisks indicate significant differences (α = 0.05, based on likelihood ratio tests) of SOM means
between 2002 and 2016 in specific soil depth layers.

236   After land use change, C and N concentrations and their isotopic compositions developed

237   differently across the soil profile. C and N concentrations increased continuously in the upper 10 cm,

238   with the accompanied depletion of the respective stable isotopes ^{13}C and ^{15}N, in particular the

239   depletion of ^{13}C in the first 5-year period (Fig. 2). In the lower layers of the former ploughing horizon

240   (10-30 cm), the concentrations of C decreased in the first ten years and increased afterwards, while N

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241   concentrations decreased in the first 5-year period and remained stable in the following nine years. In

242   the subsoil below 40 cm depth, the changes of C and N concentrations showed the same patterns as

243   in the deeper ploughing horizon, but they were less pronounced (Fig. 2). The changes of ^{13}C below the

244   upper 10 cm were relatively small and variable among the periods without a temporal direction. The

245   δ^{15}N values below the upper 10 cm slightly decreased in the first 10 years after land-use change and

246   did not change afterwards.
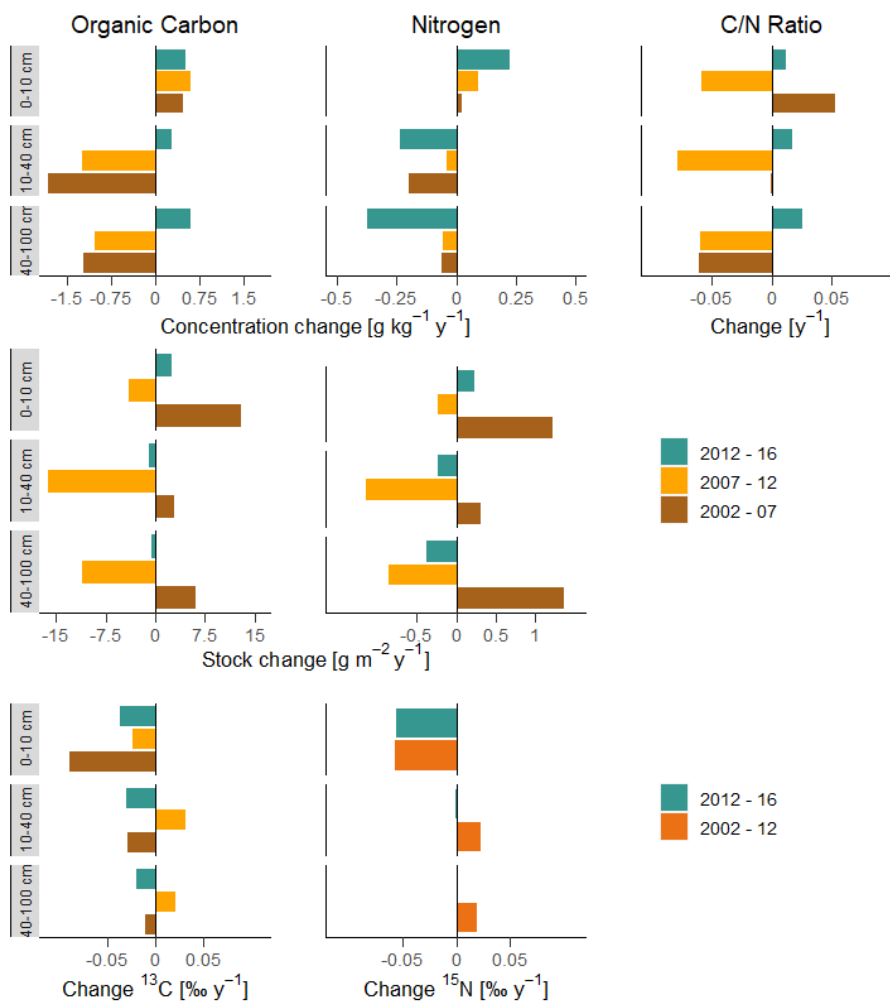
247

248   **Figure 2:** Changes of soil organic carbon and soil nitrogen concentrations and stocks, their stable isotope ratios δ^{13}C and

249   δ^{15}N values, as well as the carbon-to-nitrogen ratio (C/N ratio) at different soil depth compartments (0-10 cm, 10-40 cm, 40-

250   100 cm) during three soil sampling periods. Concentrations of the 5 cm soil increments were averaged per soil depth

251   compartments and stocks were summed up per compartment. Please note that there are no data on δ^{15}N values available

252   in 2007.

253   The changes in C and N stocks among the sampling periods were mainly driven by the high bulk

254   density observed in 2007 and the decreasing bulk densities in 2012. This pattern was observed within

255   the entire soil profile (Table S2). This resulted in increased C and N stocks in all soil depths in the first

256   period from 2002 to 2007 and a decrease of the stocks from 2007 to 2012 (Fig. 2).

Impact of plant diversity on soil organic matter changes within the soil profile

258   Changes in C and N and their isotopic composition were significantly impacted by plant

259   diversity and plant community composition at all depth increments in the first meter of the soil profile.

260   Plants impacted the concentration and stock changes in a similar way but such effects were more

261   pronounced for the concentration changes (Fig. 3). Therefore, only the significant effects (P < 0.05) on

262   concentration changes are described below. In the first 5 years after the land use change, higher plant

263   species richness led to increased C and N concentrations in the upper 5 cm of the soil (monocultures,

264   C: -0.5±2.3 g kg^{-1}, N: 0.0±0.2 g kg^{-1}; 16-species mixtures, C: +7.1±4.8 g kg^{-1}, N: 0.7±0.5 g kg^{-1}) with an

265   accompanied depletion of the ^{13}C and ^{15}N values (monocultures, Δδ^{13}C: -0.5±0.3‰, Δδ^{15}N: 0.1±0.5‰;

266   16-species mixtures, Δδ^{13}C: -1.0±0.5‰, Δδ^{15}N: -1.2±0.5‰). This positive effect of plant species

267   richness strengthened over time in the upper 5 cm and expanded over time to deeper soil layers (Fig.

268   3). Therefore, in plots with higher species richness the gains of soil C in the first 10 cm increased, while

269   the losses in the deeper ploughing horizon after the land-use change were mitigated. Specifically, the

270   plant species richness explained 27.7% and 23.8% of the changes in C and N concentrations,

271   respectively in the top 5 cm soils layer five years after the establishment of the experiment. The

272   explained variance by plant species richness increased to 54.4% and 50.2% for C and N concentrations,

273   respectively, after 14 years. Within the soil profile, the effects of plant species richness after 14 years

274   weakened with soil depth, though being significant for both C and N concentrations to the soil depths

275   of 45 and 55 cm, respectively (Fig. 3). In contrast, almost no effects were observed in soil layers below

276   the top 10 cm earlier in the experiment.

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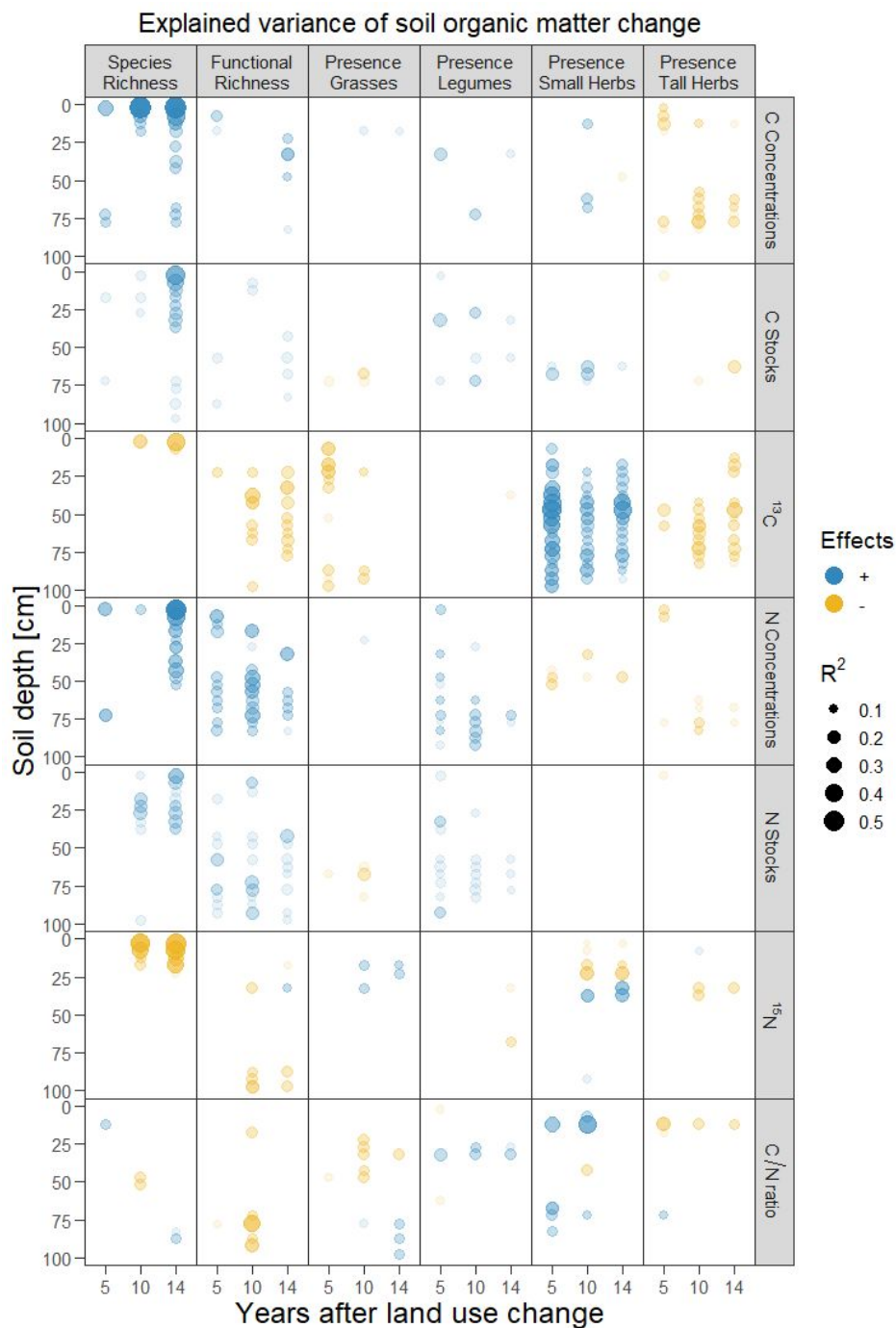
Figure 3: Explained variance (R^2) of changes in soil organic matter parameters (organic carbon and nitrogen concentrations, stocks, $\delta^{13}C$ and $\delta^{15}N$ values, and carbon to nitrogen ratio) by plant diversity and functional composition. Effects were estimated after periods of 5, 10, and 14 years. The size of the circles represents the R^2 of significant plant effects (plant species richness, functional group richness and the presence of grasses, legumes, small herbs, and tall herbs; $\alpha = 0.05$) on the respective soil parameter. Positive effects are displayed in blue, negative effects in yellow. Please note that data on $\delta^{15}N$ values were not available in 2007.

284   Similar to plant species richness, plant functional group richness positively impacted the

285   changes in soil N concentrations below the upper 10 cm, i.e., the N losses after the land-use change

286   were reduced. However, the mitigated loss of N by higher plant functional group richness was only

287   observed in the first 10 years of the experiment. On the extended 14-year period, the mitigation was

288   more driven by plant species richness (Fig. 3). Furthermore, with increased functional group richness,

289   [13]C was depleted between 20 and 75 cm, and ^{15}N was depleted between 85 and 100 cm soil depth. In

290   contrast, plant functional group richness did not affect the C concentration in soil but led to

291   increasingly depleted δ^{13}C values over time in soil depths between 25 and 75 cm.

292   Beside some transient significant effects of individual plant functional groups on the changes

293   in C concentration, tall herbs showed consistent effects. Surprisingly, C concentrations were reduced

294   in the presence of tall herbs during the first 5-year period in the top 20 cm of the soil (without tall

295   herbs: 0.0±2.3 g kg^{-1}, with tall herbs: -0.1±2.3 g kg^{-1}). Moreover, the presence of tall herbs even

296   increased C losses in the subsoil between 60 - 80 cm until the second 5-year period (without tall herbs:

297   -0.6±1.2 g kg^{-1}, with tall herbs: -1.1±1.4 g kg^{-1}). This loss of C, induced by the presence of tall herbs, was

298   accompanied with a depletion in δ^{13}C values. However, while the negative effects of the tall herbs on

299   C concentrations decreased towards the end of the study period, the depletion in δ^{13}C values became

300   stronger (without tall herbs: 0.1±0.5‰, with tall herbs: -0.3±0.4‰). The impacts of tall herbs on the

301   soil N concentration and its δ^{15}N values were similar to its negative effects on the C concentration and

302   the δ^{13}C values, but not so strong and were only significant at few individual soil depth increments.

303   The presence of small herbs led to reduced depletion in δ^{13}C values at almost the entire soil column

304   considered, while C concentrations were not significantly affected. These changes occurred mainly in

305   the first 5-year period (without small herbs: -0.5±0.4‰, with small herbs: 0.1±0.9‰) and attenuated

306   with time (difference from 2002 to 2016 without small herbs: -0.2±0.3‰, with small herbs: 0.0±0.5‰,

307   Fig. 3). Moreover, the presence of grasses depleted the δ^{13}C values in the first period; but this effect

308   declined and finally vanished over time (Fig. 3). The presence of legumes did not change δ^{13}C values.

309 In contrast, the N concentrations increased in the presence of legumes at the top 5 cm, and smaller

310 losses were found below the former ploughing horizon. In the last study period, the positive effects of

311 legume presence were only present in the depth between 70 and 80 cm (without legumes: -2.1±2.1 g

312 kg^{-1}, with legumes: -1.0±2.1 g kg^{-1}). The presence of legumes was not related to changes in δ^{15}N values,

313 indicating an overall small impact on the δ^{15}N values of the existing soil N pool.

314   C/N ratios increased strongly in the top 5 cm of the soil and decreased in the lower soil layers.

315 However, plant diversity and plant functional composition affected changes in C/N ratios only

316 sporadically and not consistently within the soil profile.

Discussion

318   Our study represents the first long-term study that elucidates the mechanisms underlying the

319 positive plant diversity effects on soil C and N storage as well as their changes over time within a soil

320 profile to 1 m depth. Using stable isotope analyses, we were able to present mechanisms underlying

321 the positive plant diversity effects on soil organic matter storage and how these effects become

322 stronger and extend to subsoil over time after land-use change.

Soil organic matter changes following the land-use change

324   This study was conducted as part of the Jena Experiment on a field site that was formerly used

325 as arable land (Roscher et al., 2004). Thus, before establishing the experiment, the soil profile of the

326 field site showed the typical distribution of soil organic matter concentrations for agricultural fields:

327 soil C and N concentrations were homogeneously distributed within the plough horizon and stocks

328 increased ongoingly towards the deeper layers of the plough horizon. Both, C and N concentrations

329 and stocks strongly decline in depth segments below the plough horizon (Angers et al., 1997). The

330 conversion from cropland to a non-fertilized experimental grassland resulted in the well-known

331 exponential decline of C and N concentrations and stocks with soil depth (e.g. Angers & Eriksen-Hamel,

332 2008; Guo & Gifford, 2002; Poeplau et al., 2011; Post & Kwon, 2000). Specifically, the increase in C and

333 N concentrations and stocks in the top 10 cm of the soil and their decline in the lower parts of the

334 plough horizon result from missing vertical mixing that caused higher biomass input to the deeper

335 layers of the plough horizon and higher root derived organic matter inputs to the top 10 cm of the soil

336 (Ravenek et al., 2014). Moreover, although changes in C and N concentrations were highly correlated

337 in all soil layers (R^2 = 0.82, Fig. S1), the C/N ratios of soil organic matter decreased below the uppermost

338 centimetres towards the C/N ratio that are typical for microbial biomass (Cleveland & Liptzin, 2007).

339 This suggests that in addition to the fresh root-derived inputs (Eisenhauer et al., 2017), other soil and

340 ecosystem parameters, like the composition and activity of micro-, meso-, and macro-decomposers

341 (Lange et al., 2015; Morriën et al., 2017; Spehn et al., 2000; Stephan et al., 2000) contribute to C and

342 N cycling and storage. Moreover, the decline of C and N concentrations and stocks in the lower plough

343 horizon and below was relatively small in most depth segments. However, in low-diversity plant

344 communities these losses were larger than the gains in the upper 10 cm of the soil (Table S2). This

345 indicates that land-use changes from croplands to low-diversity meadows might take considerably

346 longer compared to high diversity meadows until C stocks are as high as before the land-use changes

347 (Poeplau et al., 2011).

Plant diversity and community composition effects on C and N

349   Generally, concentrations of C and N reacted more sensitively to plant diversity effects and the

350 plant effects were observed earlier than in C and N stocks. This might be due to the additional

351 variability caused by physical soil effects, such as bulk density. This is in line with earlier findings

352 reporting that concentration was the most important determinant of stock variance grassland sites

353 while bulk densities were more important in croplands (Schrumpf et al., 2011).

354   The increase of both C and N concentrations and stocks within the upper 10 cm of the soil

355 profile was mainly driven by higher plant species richness, confirming results from earlier studies (Cong

356 et al., 2014; Fornara & Tilman, 2008; Lange et al., 2015). This positive plant species richness effect in

357 the top 10 cm of the soil is most likely mediated by the increased allocation of plant material to the

358 soil (Fornara & Tilman, 2008; Lange et al., 2015) in more diverse plant communities (Eisenhauer et al.,

359 2017; Ravenek et al., 2014), as indicated by the strongly depleted δ^{13}C and δ^{15}N values. This diversity

360 effect was independent of the soil texture variations across the plots (Table S3). Below the upper 10

361 cm of the soil, higher plant species richness decreased losses related to the land-use change (see

362 discussion above). These reduced losses are most likely driven by increased inputs under higher plant

363 species. For topsoil, plant diversity has been reported to increase rhizosphere C inputs into the

364 microbial community (Eisenhauer et al., 2010; Lange et al., 2015). This results in both increased

365 microbial activity and C storage as more plant-derived C is converted into forms that persists in soil

366 (Lange et al., 2015). This positive relationship between plant species richness and soil microbial activity

367 became negative in subsoil layers (Table S4). This switch indicates that the role of soil microorganisms

368 in soil organic matter dynamics changes with soil depth. Namely, in the topsoil layer, with its ample

369 supply of plant-derived C and energy, soil microorganisms may contribute to the accumulation of soil

370 organic matter (Gleixner et al., 2002; Lange et al., 2015; Liang et al., 2017), while plant inputs strongly

371 decrease with soil depth (Jobbagy & Jackson, 2000), microbial communities increasingly utilises soil

372 organic matter as energy and nutrient source. Thus, the role of microbial communities may shift

373 towards decomposition of already stored soil organic matter (Fontaine et al., 2007) within the soil

374 profile.

375   Similar effects of plant species richness on C and N concentrations were reported earlier

376 (Fornara & Tilman, 2008). However, the present study for the first time shows that changes in N

377 concentrations and δ^{15}N values are caused by different mechanisms than those driving changes in C

378 concentrations and δ^{13}C values. While increasing plant diversity typically results in higher amounts of

379 fresh plant-derived C inputs to the soil (Eisenhauer et al., 2017), only legumes are able to symbiotically

380 fix atmospheric N_2. However, in our study the presence of legumes did not explain the increases of soil

381 N concentrations or the shifts in the δ^{15}N values in the first 30 cm of the soil. This is in line with earlier

382 findings, reporting increased N storage in an experimental grassland without the presence of legumes

383 (Cong et al., 2014). However, in our study, the presence of legumes resulted in reduced losses of N in

384 subsoil, while $\delta^{15}N$ values were not significantly affected. This was in contrast to our expectations, as

385 symbiotically fixed N_2 is less discriminated in ^{15}N than inorganic N taken up by plants, e.g. as nitrate

386 (Högberg, 1997). Together with the positive effect of plant species richness on N storage within the

387 plough horizon, we suspect that plant communities with higher diversity are more effective to

388 reallocate N from deeper soil layers into upper layers by their root transport. In particular during the

389 period from 2012 to 2016, after the shift from a fertilized arable field to an unfertilized meadow (Lange

390 et al., 2019; Oelmann et al., 2011) the increasing N concentrations in the top 10 cm of the soil and the

391 decreasing below the top 10 cm (Fig. 2) together indicate a stronger N exploitation in deeper soil layers

392 and support the assumption of N reallocation.

393 Generally, we observed a shift over time of the main drivers of soil C and N dynamics from

394 plant species richness to plant functional richness or functional composition. This indicates that specific

395 functional traits related to plant functional groups impact subsoil processes. Specifically, the presence

396 of tall herbs resulted in losses of C between 60 and 80 cm soil depth, but simultaneously $\delta^{13}C$ values

397 were more depleted. This is suggestive of positive priming, a process in which decomposition of

398 existing organic C is stimulated when new C enters the system through increased microbial activity

399 (Kuzyakov, 2010). Tall herbs are considered as deep-rooting plants (Ebeling et al., 2014). Therefore, we

400 assume that fresh C from deep roots in the subsoil will eventually lead to the decomposition of already

401 stored C (Fontaine et al., 2007).

402 Small herbs did not significantly affect soil C concentrations, but in their presence $\delta^{13}C$ values

403 were less depleted in almost all soil layers below 20 cm soil depth. Due to the growth of small herbs

404 close to the soil surface, an uptake of isotopically more depleted CO_2 that derived from soil respiration

405 compared to air is likely (Roscher et al., 2011). As more depleted plant inputs would lead to a depletion

406 of the soil $\delta^{13}C$ values, the observed enrichment in $\delta^{13}C$ in the presence of small herbs indicates

407   enhanced respiration and recycling of the already stored organic matter. The enrichment in the

408   presence of small herbs was only observed in the first years after land-use change, indicating that this

409   enrichment in ^{13}C was related to the land use change.

410   The positive effect of plant species richness on soil C has been reported for short-term

411   experiments and observations (De Deyn et al., 2009; Steinbeiss et al., 2008; Xu et al., 2020). Our study

412   shows for the first time a strengthening of the positive BEF relationship for soil organic matter storage,

413   generalizing an increasing plant diversity effect over time which has mostly been reported for plant

414   biomass (e.g. Cardinale et al., 2007; Guerrero-Ramírez et al., 2017; Reich et al., 2012) but see

415   Eisenhauer et al. (2010). However, before plant species richness became the dominating driver,

416   changes in the N concentrations were more strongly driven by the richness of plant functional groups,

417   indicating complementarity between plant functional groups (Fornara & Tilman, 2008). Moreover,

418   over time, plant diversity became more important for subsoil processes, while the effects of individual

419   plant functional groups decreased in their importance. Together, this indicates that specific plant traits,

420   such as rooting depth or the ability for symbiotic fixation of N_2, drive subsoil organic matter dynamics

421   on the shorter term. In contrast, in the long-term plant species richness may become more and more

422   important as driver of subsoil organic matter dynamics. This assumption is in line with Hobley et al.

423   (2017) who proposed that organic matter storage in soils is input-driven down the whole profile; a

424   process likely driven by plant diversity over time.

425   On a global scale, the vertical distribution of root biomass is highly related to the vertical

426   distribution of soil organic C (Jobbagy & Jackson, 2000), which led to the assumption that the root

427   depth distribution drives the soil organic matter within the soil profile. In our study, however, the root

428   biomass sampled in 2014 (i.e., the latest timepoint after the land-use change in 2002) as well as the

429   root production of the years 2015 and 2016 was most strongly correlated to changes in C and N

430   concentrations during the entire period at different soil depth segments (Table S5, S6), but less to the

431   specific period of sampling. Moreover, the root biomasses of earlier years were even less strongly

432 correlated with changes in C and N concentrations in the respective time periods (Table S5). This,

433 together with the fact that the relationship between species richness and root biomass strengthens

434 with time in the Jena Experiment (Ravenek et al., 2014), raises the possibility that the root

435 development follows the soil changes after the land-use change.

436 Moreover, the positive effect of plant species richness on subsoil C and N together with the

437 negative effect of deep-rooting tall herbs on subsoil C indicate that the positive plant diversity effects

438 are not solely driven by the allocation of fresh plant-derived organic matter to deeper soil layers.

439 Instead, the results of our study suggest that plant diversity effects on soil organic matter storage start

440 at the topsoil and extend over time to deeper soil layers. This effect is most probably mediated by the

441 transport of already stored soil organic C to deeper soil layers, e.g. through bioturbation by deep-

442 burrowing earthworms, such as *Lumbricus terrestris* (Eisenhauer et al., 2009; Fischer et al., 2014). We

443 further propose that the plant diversity effect on C storage in subsoil is co-determined by the leaching

444 of soil organic matter from the topsoil and transporting it to deeper soil layers as dissolved organic

445 matter (Kaiser & Kalbitz, 2012). Although, only sporadic correlations of dissolved organic matter

446 concentrations with soil C changes were observed (Table S7), dissolved organic matter concentrations

447 themselves are highly correlated to the concentrations of overlying soil C, particular in the subsoil

448 (Table S8). This continuous transport of small amounts of organic material to deeper soil layers is likely

449 to contribute to the formation of soil organic matter (Neff & Asner, 2001). Indeed, in the Jena

450 Experiment, higher concentrations of dissolved organic matter were found with increasing plant

451 diversity (Lange et al., 2019). And although dissolved organic matter undergoes strong transformation

452 and degradation during its soil passage (Roth et al., 2019), increased concentrations of dissolved and

453 less microbially-transformed organic matter was shown to reach deeper soil layers at high plant

454 diversity (Lange et al., 2021). Thus, the spatial extension of the positive plant diversity effect on subsoil

455 organic matter highlights how grassland biodiversity can contribute to soil C sequestration (Bai &

Running title: Diversity effects on subsoil OM storage

456   Cotrufo, 2022; Yang et al., 2019) and thereby potentially mitigate the anthropogenic increase of

457   atmospheric carbon dioxide concentrations (Balesdent et al., 2018; Paustian et al., 2016).

458   The present study demonstrates that subsoil organic matter storage is significantly related to plant-

459   derived C inputs and their microbial converting to soil organic matter, in particular on longer time

460   periods. Managing plant diversity may thus have significant implications for subsoil C storage and other

461   critical ecosystem services. Moreover, the strengthening of the positive plant diversity effects on

462   organic matter storage in the topsoil and the incipient effects in subsoil indicate that new input

463   pathways of organic matter to the surface take some time to establish. After the land use changed

464   from arable land to a grassland system regular ploughing was no longer applied, and thus the

465   accompanied transfer of organic matter from the surface and topsoil to deeper soil layers stopped.

466   However, the pathways of organic matter input to the subsoil in the grassland system required first

467   the build-up of a large topsoil pool, which can then be transferred to the subsoil via DOM leaching and

468   bioturbation.

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480 **Conflict of Interest**

481 The authors declare no conflicts of interest.

482 **Data availability**

483 Data will be made publicly available after acceptance of the manuscript.

484

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